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     (FILE 'HOME' ENTERED AT 13:42:21 ON 18 FEB 2004)

     FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED
     AT 13:42:31 ON 18 FEB 2004
L1       511276 S LENTIVIR? OR HIV? OR RETROVIR?
L2          457 S (EF1-ALPHA PROMOTER) OR (PGK PROMOTER)
L3           10 S POST(L)TRANSCRIPTIONAL REGULATORY SEQUENCE
L4          125 S L1 (L) L2
L5           54 DUP REM L4 (71 DUPLICATES REMOVED)
L6           29 S L5 AND PY<=2000
L7           29 SORT L6 PY
L8           54 FOCUS L5 1-
                E TRONO DIDIER/AU
L9          127 S E3
L10         116 S L9 AND L1
L11           6 S L10 AND L2
L12           6 SORT L11 PY
L13          69 S L1 AND (WPRE OR HPRE)
L14          32 DUP REM L13 (37 DUPLICATES REMOVED)
L15           6 S L14 AND PY<=2000

     FILE 'STNGUIDE' ENTERED AT 14:09:20 ON 18 FEB 2004
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     (FILE 'HOME' ENTERED AT 13:42:21 ON 18 FEB 2004)

     FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED
     AT 13:42:31 ON 18 FEB 2004
L1        511276 S LENTIVIR? OR HIV? OR RETROVIR?
L2           457 S (EF1-ALPHA PROMOTER) OR (PGK PROMOTER)
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L6            29 S L5 AND PY<=2000
L7            29 SORT L6 PY
L8            54 FOCUS L5 1-
                 E TRONO DIDIER/AU
L9           127 S E3
L10          116 S L9 AND L1
L11            6 S L10 AND L2
L12            6 SORT L11 PY

=> d an ti so au ab pi l12 1-6

L12  ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2000:845461  CAPLUS
DN   134:141319
TI   Lentiviral gene transfer into primary and secondary NOD/SCID
     repopulating cells
SO   Blood (2000), 96(12), 3725-3733
     CODEN: BLOOAW; ISSN: 0006-4971
AU   Woods, Niels-Bjarne; Fahlman, Cecilia; Mikkola, Hanna; Hamaguchi, Isao;
     Olsson, Karin; Zufferey, Romain; Jacobsen, Sten Eirik; Trono,
     Didier; Karlsson, Stefan
AB   The ability of lentiviral vectors to transfer genes into human
     hematopoietic stem cells was studied, using a human immunodeficiency virus
     1 (HIV-1)-derived vector expressing the green fluorescence
     protein (GFP) downstream of the phosphoglycerate kinase (PGK)
     promoter and pseudotyped with the G protein of vesicular
     stomatitis virus (VSV). High-efficiency transduction of human cord blood
     CD34+ cells was achieved after overnight incubation with vector particles.
     Sixteen to 28 percent of individual colony-forming units
     granulocyte-macrophage (CFU-GM) colonies derived from cord blood CD34+
     cells were pos. by polymerase chain reaction (PCR) for the GFP gene. The
     transduction efficiency of SCID-repopulating cells (SRC) within the cord
     blood CD34+ population was assessed by serial transplantation into
     nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. When
     400 000 cord blood CD34+ cells were transplanted into primary recipients,
     all primary and secondary recipients contained and expressed the
     transgene. Over 50% of CFU-GM colonies derived from the bone marrow of
     these primary and secondary recipients contained the vector on average as
     determined by PCR. Transplantation of transduced cells in limiting dilution
     generated GFP+ lymphoid and myeloid progeny cells that may have arisen
     from a single SRC. Inverse PCR anal. was used to amplify
     vector-chromosomal junctional fragments in colonies derived from SRC and
     confirmed that the vector was integrated. These results show that
     lentiviral vectors can efficiently transduce very primitive human
     hematopoietic progenitor and stem cells.

L12  ANSWER 2 OF 6  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   2000:816778  CAPLUS
DN   135:14992
TI   High-level transgene expression in human hematopoietic progenitors and
     differentiated blood lineages after transduction with improved
     lentiviral vectors
SO   Blood (2000), 96(10), 3392-3398
     CODEN: BLOOAW; ISSN: 0006-4971
AU   Salmon, Patrick; Kindler, Vincent; Ducrey, Odile; Chapuis, Bernard;
     Zubler, Rudolf H.; Trono, Didier
AB   Recent expts. point to the great value of lentiviral vectors for
     the transduction of human hematopoietic stem cells (hHSCs). Vectors used
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so far, however, have been poorly satisfying in terms of either biosafety or efficiency of transgene expression. Herein is described the results obtained with human immunodeficiency virus-based vectors optimized in both of these aspects. It is thus shown that vectors containing the EF1α and, to a lesser extent, the phosphoglycerate kinase (**PGK**) **promoter**, govern high-level gene expression in human hematopoietic progenitors as well as derived hematopoietic lineages of therapeutic relevance, such as erythrocytes, granulocytes, monocytes, dendritic cells, and megakaryocytes. **EF1.alpha. promoter** -containing **lentiviral** vectors can also induce strong transgene expression in primary T lymphocytes isolated from peripheral blood. A self-inactivating design did not affect the performance of **EF1. alpha. promoter**-based vectors but significantly reduced expression from the **PGK promoter**. This neg. effect could nevertheless be largely rescued by inserting the post-transcriptional regulatory element of woodchuck hepatitis virus upstream of the vector 3' long terminal repeat. These results have important practical implications for the genetic treatment of lymphohematol. disorders as well as for the study of hematopoiesis via the lentivector-mediated modification of hHSCs.

L12 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:799867 CAPLUS
DN 134:361964
TI **Lentivirus** vector gene expression during ES cell-derived hematopoietic development in vitro
SO Journal of Virology (2000), 74(22), 10778-10784
CODEN: JOVIAM; ISSN: 0022-538X
AU Hamaguchi, Isao; Woods, Niels-Bjarne; Panagopoulos, Ioannis; Andersson, Elisabet; Mikkola, Hanna; Fahlman, Cecilia; Zufferey, Romain; Carlsson, Leif; **Trono, Didier**; Karlsson, Stefan
AB The murine embryonal stem (ES) cell virus (MESV) can express transgenes from the long terminal repeat (LTR) promoter/enhancer in undifferentiated ES cells, but expression is turned off upon differentiation to embryoid bodies (EBs) and hematopoietic cells in vitro. We examined whether a human immunodeficiency virus type 1-based **lentivirus** vector pseudotyped with the vesicular stomatitis virus G protein (VSV-G) could transduce ES cells efficiently and express the green fluorescent protein (GFP) transgene from an internal phosphoglycerate kinase (**PGK**) **promoter** throughout development to hematopoietic cells in vitro. An oncoretrovirus vector containing the MESV LTR and the GFP gene was used for comparison. Fluorescence-activated cell sorting anal. of transduced CCE ES cells showed 99.8 and 86.7% GPF-expressing ES cells in the VSV-G-pseudotyped **lentivirus** (multiplicity of infection [MOI] = 59)- and oncoretrovirus (MOI = 590)-transduced cells, resp. Therefore, VSV-G pseudotyping of **lentiviral** and oncoretrovirus vectors leads to efficient transduction of ES cells. **Lentivirus** vector integration was verified in the ES cell colonies by Southern blot anal. When the transduced ES cells were differentiated in vitro, expression from the oncoretrovirus LTR was severely reduced or extinct in day 6 EBs and ES cell-derived hematopoietic colonies. In contrast, many **lentivirus** -transduced colonies, expressing the GFP gene in the undifferentiated state, continued to express the transgene throughout in vitro development to EBs at day 6, and many continued to express in cells derived from hematopoietic colonies. This exptl. system can be used to analyze **lentivirus** vector design for optimal expression in hematopoietic cells and for gain-of-function expts. during ES cell development in vitro.

L12 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:52217 CAPLUS
DN 132:198941
TI Self-inactivating **lentiviral** vectors with enhanced transgene expression as potential gene transfer system in Parkinson's disease
SO Human Gene Therapy (2000), 11(1), 179-190
CODEN: HGTHE3; ISSN: 1043-0342
AU Deglon, Nicole; Tseng, Jack L.; Bensadoun, Jean-Charles; Zurn, Anne D.; Arsenijevic, Yvan; De Almeida, Luis Pereira; Zufferey, Romain; **Trono, Didier**; Aebischer, Patrick
AB Glial cell line-derived neurotrophic factor (GDNF) is able to protect

dopaminergic neurons against various insults and constitutes therefore a promising candidate for the treatment of Parkinson's disease. **Lentiviral** vectors that infect quiescent neuronal cells may allow the localized delivery of GDNF, thus avoiding potential side effects related to the activation of other brain structures. To test this hypothesis in a setting ensuring both maximal biosafety and optimal transgene expression, a self-inactivating (SIN) **lentiviral** vector was modified by insertion of the posttranscriptional regulatory element of the woodchuck hepatitis virus, and particles were produced with a multiply attenuated packaging system. After a single injection of 2 μl of a lacZ-expressing vector (SIN-W-LacZ) in the substantia nigra of adult rats, an average of 40.1 ± 6.0% of the tyrosine hydroxylase (TH)-pos. neurons were transduced as compared with 5.0 ± 2.1% with the first-generation **lentiviral** vector. Moreover, the SIN-W vector expressing GDNF under the control of the mouse phosphoglycerate kinase 1 (**PGK**) **promoter** was able to protect nigral dopaminergic neurons after medial forebrain bundle axotomy. Expression of hGDNF in the nanogram range was detected in exts. of mesencephalon of animals injected with an SIN-W-PGK-GDNF vector, whereas it was undetectable in animals injected with a control vector. **Lentiviral** vectors with enhanced expression and safety features further establish the potential use of these vectors for the local delivery of bioactive mols. into defined structures of the central nervous system.

L12 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:117973 CAPLUS
DN 138:164686
TI Highly contained replication incompetent **lentiviral** gene therapy vectors and systems for their propagation
SO PCT Int. Appl., 94 pp.
CODEN: PIXXD2
IN **Trono, Didier**; Zufferey, Romain N.
AB **Lentivirus** vectors derived from human immunodeficiency virus that have a number of modifications that make them very safe, efficient, high-level expression vectors for gene therapy are described. The modifications include, in combination: an inactive central polypurine tract, a stuffer sequence, which may encode drug susceptibility genes, and a mutated hairpin in the 5' leader sequence that substantially abolishes replication. In addition, genes essential for viral replication are on plasmids containing mutations that prevent replication competent virus being formed by recombination. These elements are provided in conjunction with other features of **lentiviral** vectors, such as a self-inactivating configuration for biosafety and promoters such as the **EF1.alpha. promoter** as one example. Addnl. promoters are also described. The vectors can also comprise addnl. transcription enhancing elements such as the wood chuck hepatitis virus post-transcriptional regulatory element. These vectors therefore provide useful tools for genetic treatments for inherited and acquired disorders, gene-therapies for cancers and other disease, the creation of industrial and exptl. production systems utilizing transformed cells, as well as for the study of basic cellular and genetic processes.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2003012054	A2	20030213	WO 2002-US24275	20020801
WO 2003012054	A3	20031120		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2003082789	A1	20030501	US 2002-209952	20020801

L12 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:23440 CAPLUS
DN 138:84478
TI Self-inactivating **lentiviral** vectors for gene therapy capable of driving high level expression of therapeutic genes
SO U.S. Pat. Appl. Publ., 40 pp.
CODEN: USXXCO
IN **Trono, Didier**; Salmon, Patrick
AB **HIV**-derived **lentivirus** vectors which are safe, highly efficient, and drive high levels of expression of transgenes in human cells for gene therapy, especially, in human hematopoietic progenitor cells as well as in all other blood cell derivs. are described. The **lentiviral** vectors comprise a self-inactivating configuration for biosafety. The vectors carry only the gag, pol, and rev genes. The promoter function of the long terminal repeats (LTR) is diminished by inactivation of the U3 region of the right LTR. Promoters such as the **EF1.alpha**. **promoter** are used to drive transgene expression and addnl. promoters are also described. The vectors can also comprise addnl. transcription enhancing elements such as the wood chuck hepatitis virus post-transcriptional regulatory element. These vectors therefore provide useful tools for genetic treatments such as inherited and acquired lympho-hematol. disorders, gene-therapies for cancers especially the hematol. cancers, as well as for the study of hematopoiesis via lentivector-mediated modification of human HSCs. Construction of vectors based on **HIV**-1 and murine leukemia virus is demonstrated. Vectors pseudotyped with vesicular stomatitis virus G glycoproteins efficiently infected CD34+ cells. Efficient expression of reporter genes from PGK and **EF1.alpha**. **promoters** was seen.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003008374	A1	20030109	US 2001-10081	20011109

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L15 ANSWER 3 OF 6 MEDLINE on STN
AN 1999173983 MEDLINE
TI Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by **retroviral** vectors.
SO JOURNAL OF VIROLOGY, **(1999 Apr)** 73 (4) 2886-92.
Journal code: 0113724. ISSN: 0022-538X.
AU Zufferey R; Donello J E; Trono D; Hope T J
AB The expression of genes delivered by **retroviral** vectors is often inefficient, a potential obstacle for their widespread use in human gene therapy. Here, we explored the possibility that the posttranscriptional regulatory element of woodchuck hepatitis virus (**WPRE**) might help resolve this problem. Insertion of the **WPRE** in the 3' untranslated region of coding sequences carried by either oncoretroviral or **lentiviral** vectors substantially increased their levels of expression in a transgene-, promoter- and vector-independent manner. The **WPRE** thus increased either luciferase or green fluorescent protein production five- to eightfold, and effects of a comparable magnitude were observed with either the immediate-early cytomegalovirus or the herpesvirus thymidine kinase promoter and with both human immunodeficiency virus- and murine leukemia virus-based vectors. The **WPRE** exerted this influence only when placed in the sense orientation, consistent with its predicted posttranscriptional mechanism of action. These results demonstrate that the **WPRE** significantly improves the performance of **retroviral** vectors and emphasize that posttranscriptional regulation of gene expression should be taken into account in the design of gene delivery systems.

L15 ANSWER 4 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 1999:1156 SCISEARCH
TI The hepatitis virus post transcriptional RNA export element (**HPRE**) can increase expression from **retroviral** vectors and may improve the transport of intronless RNA of the normally intron-dependent B-globin cDNA.
SO BLOOD, (**15 NOV 1998**) Vol. 92, No. 10, Part 1, Supp. [1], pp. 587-587.
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399.
ISSN: 0006-4971.
AU Jiang G (Reprint); Mathias L A; Xu D; Pepper K; Malik P

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L2           457 S (EF1-ALPHA PROMOTER) OR (PGK PROMOTER)
L3            10 S POST(L)TRANSCRIPTIONAL REGULATORY SEQUENCE
L4           125 S L1 (L) L2
L5            54 DUP REM L4 (71 DUPLICATES REMOVED)
L6            29 S L5 AND PY<=2000
L7            29 SORT L6 PY

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L7   ANSWER 25 OF 29     MEDLINE on STN
AN   2000110569     MEDLINE
TI   Self-inactivating lentiviral vectors with enhanced transgene expression as
     potential gene transfer system in Parkinson's disease.
SO   HUMAN GENE THERAPY, (2000 Jan 1) 11 (1) 179-90.
     Journal code: 9008950. ISSN: 1043-0342.
AU   Deglon N; Tseng J L; Bensadoun J C; Zurn A D; Arsenijevic Y; Pereira de
     Almeida L; Zufferey R; Trono D; Aebischer P
AB   Glial cell line-derived neurotrophic factor (GDNF) is able to protect
     dopaminergic neurons against various insults and constitutes therefore a
     promising candidate for the treatment of Parkinson's disease.
     Lentiviral vectors that infect quiescent neuronal cells may allow
     the localized delivery of GDNF, thus avoiding potential side effects
     related to the activation of other brain structures. To test this
     hypothesis in a setting ensuring both maximal biosafety and optimal
     transgene expression, a self-inactivating (SIN) lentiviral
     vector was modified by insertion of the posttranscriptional regulatory
     element of the woodchuck hepatitis virus, and particles were produced with
     a multiply attenuated packaging system. After a single injection of 2
     microl of a lacZ-expressing vector (SIN-W-LacZ) in the substantia nigra of
     adult rats, an average of 40.1 +/- 6.0% of the tyrosine hydroxylase
     (TH)-positive neurons were transduced as compared with 5.0 +/- 2.1% with
     the first-generation lentiviral vector. Moreover, the SIN-W
     vector expressing GDNF under the control of the mouse phosphoglycerate
     kinase 1 (PGK) promoter was able to protect nigral
     dopaminergic neurons after medial forebrain bundle axotomy. Expression of
     hGDNF in the nanogram range was detected in extracts of mesencephalon of
     animals injected with an SIN-W-PGK-GDNF vector, whereas it was
     undetectable in animals injected with a control vector.
     Lentiviral vectors with enhanced expression and safety features
     further establish the potential use of these vectors for the local
     delivery of bioactive molecules into defined structures of the central
     nervous system.

L7   ANSWER 20 OF 29     MEDLINE on STN
AN   2001076356     MEDLINE
TI   High-level transgene expression in human hematopoietic progenitors and
     differentiated blood lineages after transduction with improved lentiviral
     vectors.
SO   BLOOD, (2000 Nov 15) 96 (10) 3392-8.
     Journal code: 7603509. ISSN: 0006-4971.
AU   Salmon P; Kindler V; Ducrey O; Chapuis B; Zubler R H; Trono D
AB   Recent experiments point to the great value of lentiviral
     vectors for the transduction of human hematopoietic stem cells (hHSCs).
     Vectors used so far, however, have been poorly satisfying in terms of
     either biosafety or efficiency of transgene expression. Herein is
     described the results obtained with human immunodeficiency virus-based
     vectors optimized in both of these aspects. It is thus shown that vectors
     containing the EF1alpha and, to a lesser extent, the phosphoglycerate
     kinase (PGK) promoter, govern high-level gene
     expression in human hematopoietic progenitors as well as derived
     hematopoietic lineages of therapeutic relevance, such as erythrocytes,
     granulocytes, monocytes, dendritic cells, and megakaryocytes. EF1alpha
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promoter-containing **lentiviral** vectors can also induce strong transgene expression in primary T lymphocytes isolated from peripheral blood. A self-inactivating design did not affect the performance of EF1alpha promoter-based vectors but significantly reduced expression from the **PGK promoter**. This negative effect could nevertheless be largely rescued by inserting the post-transcriptional regulatory element of woodchuck hepatitis virus upstream of the vector 3' long terminal repeat. These results have important practical implications for the genetic treatment of lymphohematologic disorders as well as for the study of hematopoiesis via the lentivector-mediated modification of hHSCs.

L7 ANSWER 26 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 2000:462961 SCISEARCH
TI Plat-E: an efficient and stable system for transient packaging of retroviruses
SO GENE THERAPY, (**JUN 2000**) Vol. 7, No. 12, pp. 1063-1066.
Publisher: NATURE PUBLISHING GROUP, HOUNDMILLS, BASINGSTOKE RG21 6XS, HAMPSHIRE, ENGLAND.
ISSN: 0969-7128.
AU Morita S; Kojima T; Kitamura T (Reprint)
AB A potent **retrovirus** packaging cell line named Platinum-E (Plat-E) was generated based on the 293T cell line. Plat-E is superior to existing packaging cell lines regarding efficiency, stability and safety. The novel packaging constructs utilized in establishment of Plat-E ensure high and stable expression of viral structural proteins. Conventional packaging constructs made use of the promoter of MuLV-LTR for expression of viral structural genes gag-pol and env, while our packaging constructs utilized the **EF1 alpha promoter**, which is 100-fold more potent than the MuLV-LTR in 293T cells in combination with the Kozak's consensus sequence upstream of the initiation codon resulting in high expression of virus structural proteins in Plat-E cells. To maintain the high titers of **retroviruses** under drug selection pressure, we inserted the IRES (internal ribosome entry site) sequence between the gene encoding gag-pol or env, and the gene encoding a selectable marker in the packaging constructs. Plat-E cells can stably produce **retroviruses** with an average titer of 1 x 10(7)/ml for at least 4 months. In addition, as we used only the coding sequences of viral structural genes to avoid inclusion of unnecessary **retrovirus** sequences in the packaging constructs, the probability of generating the replication competent **retroviruses** (RCR) by recombination can virtually be ruled out.

L7 ANSWER 24 OF 29 MEDLINE on STN
AN 2000468352 MEDLINE
TI Gene transfer to hepatocellular carcinoma: transduction efficacy and transgene expression kinetics by using retroviral and lentiviral vectors.
SO CANCER GENE THERAPY, **(2000 Sep)** 7 (9) 1286-92.
Journal code: 9432230. ISSN: 0929-1903.
AU Gerolami R; Uch R; Jordier F; Chapel S; Bagnis C; Brechot C; Mannoni P
AB Gene therapy is an attractive therapy for hepatocarcinoma, and several approaches have been studied using murine leukemia virus-derived **retroviruses**. We compared gene transfer efficacy and transgene expression kinetics after transduction of hepatocarcinoma cell lines using enhanced green fluorescent protein (EGFP)-expressing murine leukemia virus-derived **retroviral** vectors and **HIV**-derived **lentiviral** vectors. First, we showed that both **retroviral** and **lentiviral** vectors efficiently transduce cycling hepatocarcinoma cell lines in vitro. However, after cell cycle arrest, transduction efficacy remained the same for **lentiviral** vectors but it decreased by 80% for **retroviral** vectors. Second, we studied EGFP expression kinetics using **lentiviral** vectors expressing EGFP under the control of cytomegalovirus (CMV) or phosphoglycerolkinase (**PGK**) **promoter**. We show that the CMV promoter allows a stronger EGFP expression than the **PGK promoter**. However, in contrast to PGK-driven EGFP expression, which persists up to 2 months after transduction, CMV-driven EGFP expression rapidly decreased with time. This phenomenon is due to promoter silencing, and EGFP expression can be restored in transduced

cells by using transcription activators such as interleukin-6 or phorbol myristate acetate/ionomycin and, to a lesser extent, the demethylating agent 5'-azacytidine. Altogether, our results suggest that **lentiviral** vectors, which allow efficient transduction of hepatocarcinoma cell lines with a strong and a sustained expression according to the promoter used, are promising tools for gene therapy of hepatocarcinomas.

L7 ANSWER 21 OF 29 MEDLINE on STN
AN 2001064792 MEDLINE
TI Retroviral vector design studies toward hematopoietic stem cell gene therapy for mucopolysaccharidosis type I.
SO GENE THERAPY, **(2000 Nov)** 7 (21) 1875-83.
Journal code: 9421525. ISSN: 0969-7128.
AU Pan D; Aronovich E; McIvor R S; Whitley C B
AB To optimize a gene transfer system for hematopoietic stem cell gene therapy of patients with mucopolysaccharidosis (MPS) type I, 10 **retroviral** vectors were constructed to express the human alpha-L-iduronidase (IDUA) cDNA. These vectors were designed to evaluate the potential effects of specific promoters, the addition of selectable markers, and the use of multiple promoters versus an internal ribosome entry site for expression of IDUA and selectable maker genes. The effect of vector design was investigated in primary patient fibroblasts (F(MPS)) or murine fibroblast cell lines; while overall comparison of transgene expression was determined in patients' peripheral blood lymphocytes (PBL(MPS)) and CD34+ progenitors (PBPC(MPS)). We observed that the human **PGK promoter** introduced the highest IDUA activity per 1% relative transgene frequency in F(MPS). Use of the same promoter to separately regulate both the therapeutic gene and a drug-resistance gene resulted in decreased expression of the unselected gene. Co-selection using bicistronic vectors not only increased the number of transductants, but also elevated transgene expression under selective pressure in transgene-positive progenitors. Bicistronic vector LP1CD overcame down-regulation and practically introduced the highest IDUA level in unselected PBL(MPS) and an intermediate level in PBPC(MPS). These studies provide a better understanding of factors contributing to efficient gene expression in hematopoietic cells.

L7 ANSWER 19 OF 29 MEDLINE on STN
AN 2001096196 MEDLINE
TI Lentiviral gene transfer into primary and secondary NOD/SCID repopulating cells.
SO BLOOD, **(2000 Dec 1)** 96 (12) 3725-33.
Journal code: 7603509. ISSN: 0006-4971.
AU Woods N B; Fahlman C; Mikkola H; Hamaguchi I; Olsson K; Zufferey R; Jacobsen S E; Trono D; Karlsson S
AB The ability of **lentiviral** vectors to transfer genes into human hematopoietic stem cells was studied, using a human immunodeficiency virus 1 (**HIV**-1)-derived vector expressing the green fluorescence protein (GFP) downstream of the phosphoglycerate kinase (**PGK**) **promoter** and pseudotyped with the G protein of vesicular stomatitis virus (VSV). High-efficiency transduction of human cord blood CD34(+) cells was achieved after overnight incubation with vector particles. Sixteen to 28 percent of individual colony-forming units granulocyte-macrophage (CFU-GM) colonies derived from cord blood CD34(+) cells were positive by polymerase chain reaction (PCR) for the GFP gene. The transduction efficiency of SCID-repopulating cells (SRC) within the cord blood CD34(+) population was assessed by serial transplantation into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. When 400,000 cord blood CD34(+) cells were transplanted into primary recipients, all primary and secondary recipients contained and expressed the transgene. Over 50% of CFU-GM colonies derived from the bone marrow of these primary and secondary recipients contained the vector on average as determined by PCR. Transplantation of transduced cells in limiting dilution generated GFP(+) lymphoid and myeloid progeny cells that may have arisen from a single SRC. Inverse PCR analysis was used to amplify vector-chromosomal junctional fragments in colonies derived from SRC and confirmed that the vector was integrated. These results show that **lentiviral** vectors can efficiently transduce very primitive human

hematopoietic progenitor and stem cells. (Blood. 2000;96:3725-3733)

L7 ANSWER 18 OF 29 MEDLINE on STN
AN 2001096512 MEDLINE
TI Lentiviral vectors for enhanced gene expression in human hematopoietic cells.
SO MOLECULAR THERAPY, **(2000 Nov)** 2 (5) 458-69.
Journal code: 100890581. ISSN: 1525-0016.
AU Ramezani A; Hawley T S; Hawley R G
AB Accumulated data indicate that current generation **lentiviral** vectors, which generally utilize an internal human cytomegalovirus (CMV) immediate early region enhancer-promoter to transcribe the gene of interest, are not yet optimized for efficient expression in human hematopoietic stem/progenitor cells (HSPCs). As a first step toward this goal, we constructed self-inactivating derivatives of the **HIV** -1-based transfer vector pHR' containing the enhanced green fluorescent protein (GFP) gene as reporter and the Woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). GFP expression was driven by a variety of strong viral and cellular promoters, including the murine stem cell virus (MSCV) long terminal repeat (LTR), a Gibbon ape leukemia virus (GALV) LTR, the human elongation factor 1alpha (EF1alpha) promoter, the composite CAG promoter (consisting of the CMV immediate early enhancer and the chicken beta-actin promoter), and the human phosphoglycerate kinase 1 (**PGK**) **promoter**. In contrast to results obtained in human embryonic kidney 293T cells and fibrosarcoma HT1080 cells, in which the CMV promoter expressed GFP at the highest levels, significantly higher levels of GFP expression (3- to 5-fold) were achieved with the MSCV LTR, the EF1alpha promoter, and the CAG promoter in the human HSPC line KG1a. Removal of the WPRE indicated that it stimulated GFP expression from all of the vectors in KG1a cells (up to 3-fold), although it only marginally improved the performance of the intron-containing EF1alpha and CAG promoters (<1.5-fold stimulation). The vectors using the MSCV LTR, the GALV LTR, and the **PGK promoter** were the most efficient at transducing primary human CD34+ cord blood progenitors under the conditions employed. High-level GFP expression in the NOD/SCID xenograft model was demonstrated with the pHR' derivative bearing the MSCV LTR. These new **lentiviral** vector backbones provide a basis for the rational design of improved delivery vehicles for human HSPC gene transfer applications.

L7 ANSWER 17 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 1999:244059 SCISEARCH
TI Regulation of N-acetylgalactosamine 4-sulfatase expression in retrovirus-transduced feline mucopolysaccharidosis type VI muscle cells
SO DNA AND CELL BIOLOGY, (**MAR 1999**) Vol. 18, No. 3, pp. 187-195.
Publisher: MARY ANN LIEBERT INC PUBL, 2 MADISON AVENUE, LARCHMONT, NY 10538.
ISSN: 1044-5498.
AU Yogalingam G (Reprint); Muller V; Hopwood J J; Anson D S
AB As a preliminary step toward muscle-mediated gene therapy in the mucopolysaccharidosis (MPS) type VI cat, we have analyzed the transcriptional regulation of feline N-acetylgalactosamine 4-sulfatase (f4S) gene expression from various **retroviral** constructs in primary cultures of muscle cells. Two **retroviral** constructs were made containing the f4S cDNA under the transcriptional control of the human polypeptide chain-elongation factor 1 alpha (EF1 alpha) gene promoter or the cytomegalovirus (CMV) immediate-early promoter. Two further **retroviral** constructs were made with the murine muscle creatine kinase (mck) enhancer sequence upstream of the internal promoter. Virus made from each construct was used to transduce feline MPS VI myoblasts. The mck enhancer significantly upregulated f4S gene expression from both the **EF1 alpha promoter** and the CMV promoter in transduced myoblasts and in differentiated myofibers. The highest level of 4S activity was observed in myoblasts and myofibers transduced with the **retroviral** construct L<(mck)under bar>cmv4S, in which the f4S gene is under the transcriptional regulation of the mck enhancer and CMV immediate-early promoter. L<(mck)under bar>cmv4S-transduced myofibers demonstrated correction of glycosaminoglycan storage and contained a 58-fold elevated level of 4S

activity compared with normal myofibers. Recombinant f4S secreted from L<(mck)under bar>cmv4S-transduced myofibers was endocytosed by feline MPS VI myofibers, leading to correction of the biochemical storage phenotype.

L7 ANSWER 15 OF 29 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1997:207663 CAPLUS
DN 126:196108
TI Retrovirus gene therapy that self-inactivate by sequence-specific recombination
SO Ger. Offen., 10 pp.
CODEN: GWXXBX
IN von Melchner, Harald; Grez, Manuel; Russ, Andreas Peter
AB **Retroviral** gene therapy vectors that eliminate sequences not associated with the therapeutic expression cassette after integration into the target cell are described. The elimination of non-essential sequences from the target cell helps to avoid drawbacks associated with the use of **retroviral** vectors, such as the activation of protooncogenes. The elimination of these sequences is brought about by incorporating a site-specific recombinase system into the vector. The construction of a Moloney murine leukemia virus expression vector with a Cre recombinase gene under control of the **pgk promoter** incorporated into the U3 region of the 5'-LTR is described. The viral genome also included a pair of loxP elements. Successful deletion of the sequence between the loxP sites was demonstrated in transfected NIH3T3 cells.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 19530412	A1	19970220	DE 1995-19530412	19950818 <--
WO 9707223	A1	19970227	WO 1996-EP761	19960223 <--
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR				
AU 9649410	A1	19970312	AU 1996-49410	19960223 <--
EP 845041	A1	19980603	EP 1996-905788	19960223 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE				
JP 11511018	T2	19990928	JP 1996-508854	19960223 <--

L7 ANSWER 13 OF 29 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 96:566742 SCISEARCH
TI CONSTRUCTION OF YEAST VECTORS POTENTIALLY USEFUL FOR EXPRESSION OF EUKARYOTIC GENES AS BETA-GALACTOSIDASE FUSION PROTEINS
SO JOURNAL OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, (**31 JUL 1996**) Vol. 29, No. 4, pp. 359-364.
ISSN: 1225-8687.
AU CHUNG K S (Reprint); CHOI W J; LEE H W; KIM K W; YOO H S
AB By both in vitro hydroxylamine mutagenesis of the wild type 3-phosphoglycerate kinase gene (**PGK**) **promoter** DNA and insertion of the leu2-d gene, we have created yeast expression vectors potentially useful for production of eukaryotic genes in yeast. The guanine (G) to adenine (A) change at the -3 position from the ATG start codon of the **PGK promoter**-based vector rendered a 6 similar to 7 times elevated expression of the adjacent eukaryotic gene, and insertion of the leu2-d gene in the vector containing the mutated **PGK promoter** further enhanced the expression of the gene. When expression of the AIDS virus **HIV1**-gagP17 gene in a lacZ fusion form was examined with this new vector, a 15 times higher level of expression than that from the original **PGK promoter** was observed. Northern and Southern analysis showed that this elevated expression is due to the production of a high copy number of mRNA by leu2-d gene functioning and by efficient translation of the produced mRNA. Thus, the vector that contained the A at the -3 position from the ATG start codon in the promoter region and the leu2-d gene shows increased expression capability and will be potentially useful for production of eukaryotic genes in yeast.

L7 ANSWER 9 OF 29 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1996:378822 CAPLUS
DN 125:50293
TI Studies on tumor gene therapy by the HSV-tk/ACV system
SO Fudan Xuebao, Ziran Kexueban (**1995**), 34(6), 691-699
CODEN: FHPTAY; ISSN: 0427-7104
AU Lu, Daru; Pan, Yujun; Zhang, Hong; Wang Hongwei; Xue, Jinglun; Qiu, Xinfang
AB **Retroviral** vector pLNTK carrying herpes simplex virus (HSV) tk gene driven by **pgk promoter** was constructed and transferred into human glioma cell SHG44 and mice melanoma cell B16 by **retroviral** transfection. The gene transferred cells were designated as SHGLNTK and B16NTK resp. In in vitro study, acyclovir (ACV) sensitive levels of tk expressed cells were significantly higher than that of their parent cells. ACV sensitive levels of SHGLNTK and B16NTK were 1000 and 400 times of their parent cells, resp. The increment of ACV sensitive level of parent cells coculturing with gene modified cells suggested the presence of by-stander effects. In in vivo study, SHGLNTK cells were sensitive to ACV treatment and could be eliminated completely when ACV was administered to BVALB/C nude mice. When the tumor had grown to 1 cm diameter, after treatment with ACV, the volume of SHGLNTK tumor decreased to 20% of the origin size. B16LNTK cells were sensitive to ACV as well, the tumor volume of B16LNTK is decreased to only 5% of the volume of B16 in C57/BL mice. Mice carrying tumor induced by the implantation of SHG44 or B16 cells were in situ with injection of 200 μl of **retroviral** supernatant (titer for 2+104 CfU) or implantation of PA317 cells (1+107) which produced **retroviral** vector LNTK. Tumor volume of B16 treated with **retrovirus** supernatant decreased to 57% of his origin. Domestic production of ACV showed no difference with that made in England. The HSV-tk/ACV system is a promising assay for tumor gene therapy.

L7 ANSWER 3 OF 29 MEDLINE on STN
AN 94362229 MEDLINE
TI Retroviral vector design for long-term expression in murine hematopoietic cells in vivo.
SO BLOOD, **(1994 Sep 15)** 84 (6) 1812-22.
Journal code: 7603509. ISSN: 0006-4971.
AU Correll P H; Colilla S; Karlsson S
AB A series of **retroviral** vectors containing the human glucocerebrosidase (GC) cDNA driven by various promoters have been constructed in an attempt to discover which vector design can most efficiently transduce murine hematopoietic stem cells (HSCs) and drive expression of the transferred gene in hematopoietic cells of mice reconstituted with the transduced stem cells. The simplest vector, LG, in which the GC gene is driven by the viral LTR, was the most efficient vector at infecting HSCs, with an average viral copy number in hematopoietic tissues of 3 copies/cell in recipient mice. In general, the viral vectors that contained any additional promoters or enhancers to drive expression of either the GC gene or a selectable marker gene (Neo) had lower titers and/or transduced HSCs at a lower efficiency. This was seen most markedly when the human phosphoglycerate (**PGK**) **promoter** was used to drive the human GC cDNA. Despite repeated attempts to obtain a high titer producer clone, this virus consistently produced low titers and subsequently resulted in the lowest proviral copy numbers in long-term reconstituted mice. Only the viral LTR and **PGK promoter** were capable of driving significant levels of human GC RNA in hematopoietic cells of long-term reconstituted mice, with a much lower level of RNA generated by an internal herpes TK or SV40 immediate early promoter. Insertion of the internal transcription unit in the opposite orientation relative to the viral LTRs had a detrimental effect on gene expression. The levels of RNA generated by a hybrid LTR containing the myeloproliferative sarcoma virus enhancer were higher in bone marrow-derived macrophages than in nonadherent cells of the bone marrow when compared with the LG vector. The presence of an internal promoter to drive expression of the human GC cDNA did not seem to have a detrimental effect on expression levels from the viral LTR. In fact, in the presence of an internal TK or **PGK promoter** expression from the LTR was increased despite the presence of lower proviral copy numbers. Insertion of a second gene (Neo) into the vector

had a negative impact on long-term expression in hematopoietic cells in vivo; however, this seems to be due solely to the lower transduction efficiency of this vector. Overall, the highest levels of GC activity in macrophages of long-term reconstituted mice were generated by the LG vector; however, these levels were variable.(ABSTRACT TRUNCATED AT 400 WORDS)

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STN: SEARCH HISTORY